OMTO, Volume *24*

Supplemental information

A novel TanCAR targeting IL13Rα2 and EphA2 for enhanced glioblastoma therapy

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Supplemental Information

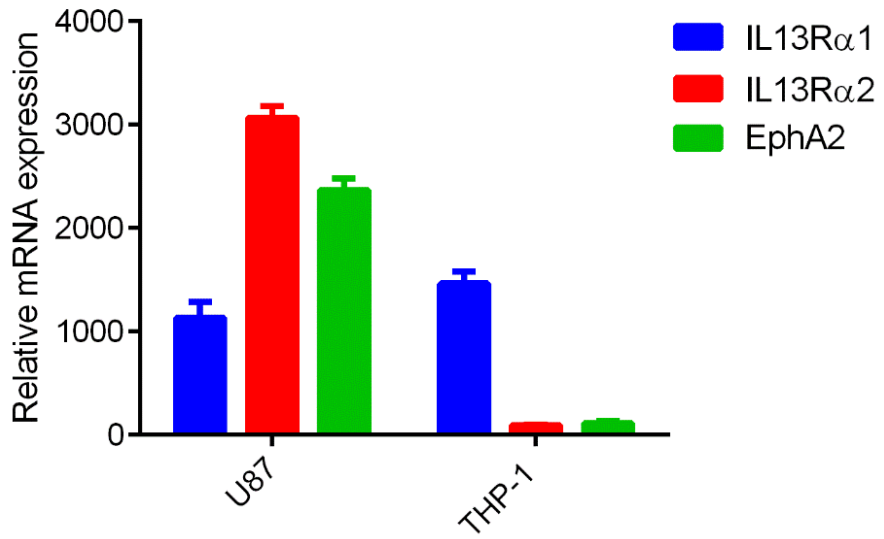

Figure S1. The relative mRNA expression level of EphA2, IL13Rα1, and IL13Rα2 genes in U87 and THP-1 cell lines.

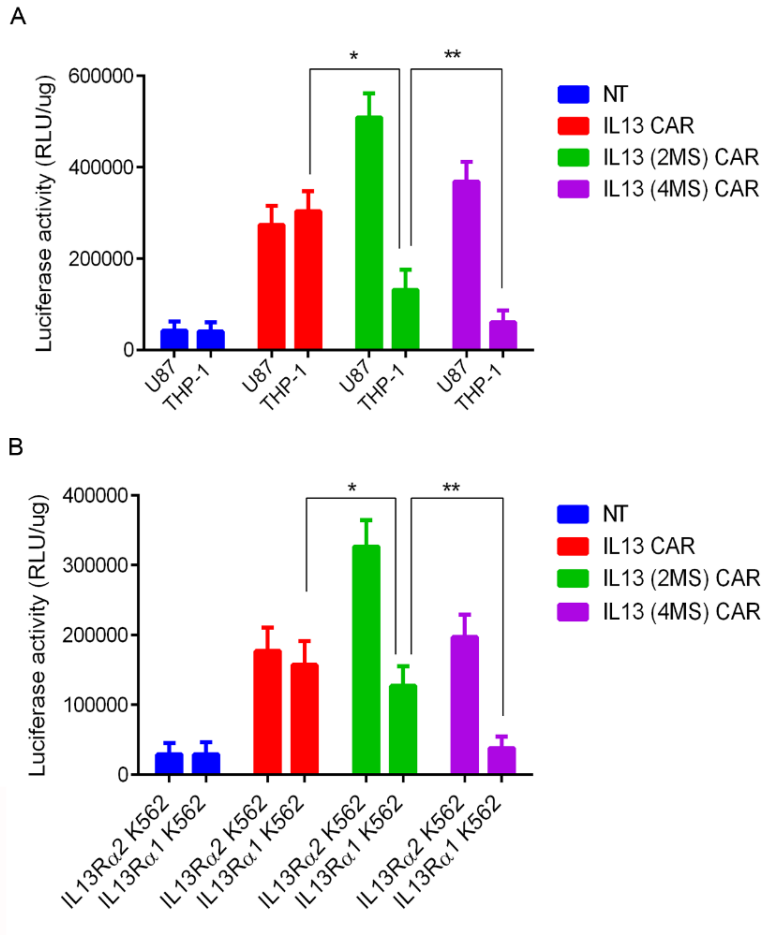

Figure S2. Functional characterization of the novel IL13 CAR in Jurkat T cells. (A) Non-transduced (NT) Jurkat T cells, IL13 Jurkat CAR-T cells, IL13 (2MS) Jurkat CAR-T cells, and IL13 (4MS) Jurkat CAR-T cells were co-cultured with U87 cells or THP-1 cells at an effector to target (E:T) ratio of 1×10^5:1×10^5, and after 24 hours, luciferase activity was measured using a luciferase reporter assay kit. **(B)** NT Jurkat T cells, IL13 Jurkat CAR-T cells, IL13 (E13K.R109K) Jurkat CAR-T cells, and IL13 (4MS) Jurkat CAR-T cells were co-cultured with IL13Rα2- or IL13Rα1-engineered K562 target cells at an E:T ratio of 1×10^5:1×10^5, and after 24 hours, luciferase activity was measured using a luciferase reporter assay kit and a Varioskan Flash multitechnology microplate reader. Statistically significant differences are indicated: *P<0.05; **P<0.01. IL13 (2MS) stands for IL13 (E13K.R109K), while IL13 (4MS) stands for IL13 (E13K.R66D.S69D.R109K).

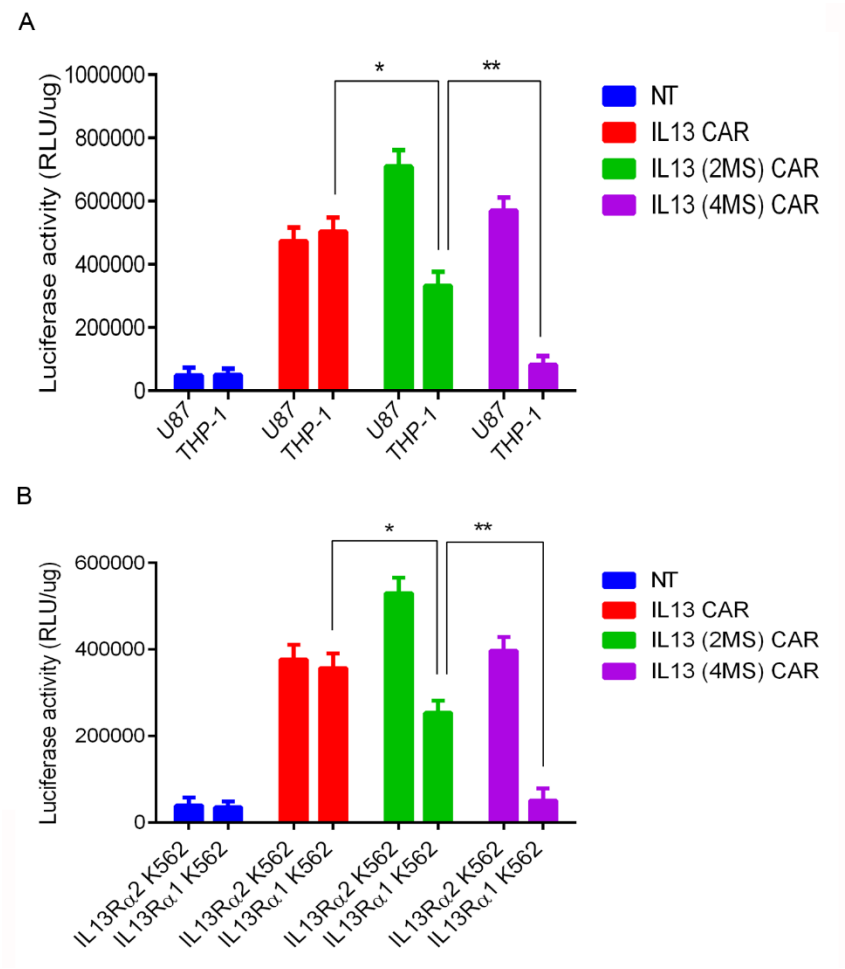

Figure S3. Functional characterization of the novel IL13 CAR in Jurkat T cells. (A) Non-transduced (NT) Jurkat T cells, IL13 Jurkat CAR-T cells, IL13 (2MS) Jurkat CAR-T cells, and IL13 (4MS) Jurkat CAR-T cells were co-cultured with U87 cells or THP-1 cells at an effector to target (E:T) ratio of 1×10^5:1×10^5, and after 24 hours, luciferase activity was measured using a luciferase reporter assay kit. **(B)** NT Jurkat T cells, IL13 Jurkat CAR-T cells, IL13 (E13K.R109K) Jurkat CAR-T cells, and IL13 (4MS) Jurkat CAR-T cells were co-cultured with IL13Rα2- or IL13Rα1-engineered K562 target cells at an E:T ratio of 1×10^5:1×10^5, and after 24 hours, luciferase activity was measured using a luciferase reporter assay kit and a Varioskan Flash multitechnology microplate reader. Statistically significant differences are indicated: *P<0.05; **P<0.01. IL13 (2MS) stands for IL13 (E13K.R109K), while IL13 (4MS) stands for IL13 (E13K.R66D.S69D.R109K).

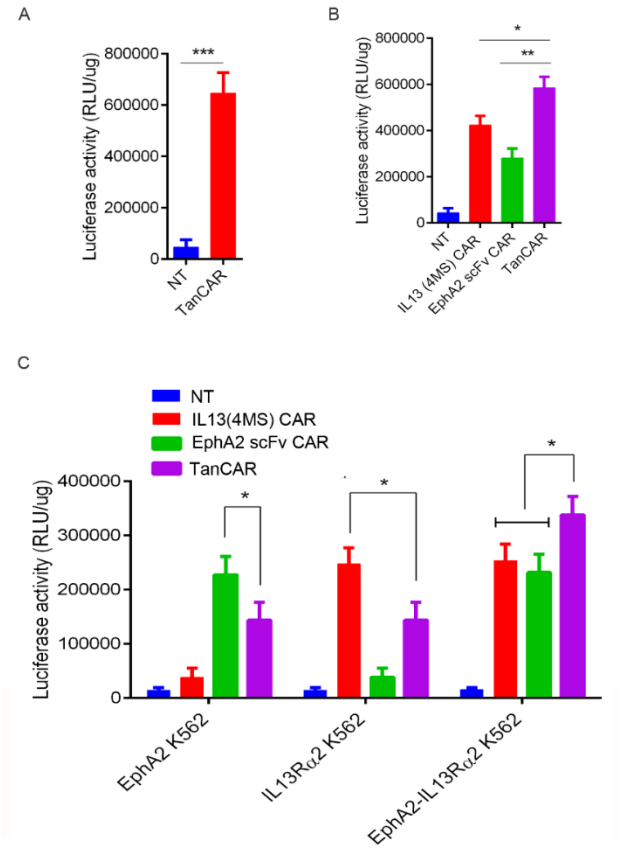

Figure S4. Functional characterization of the novel bispecific IL13 (4MS)-EphA2 scFv-TanCAR in Jurkat T cells. (A) NT Jurkat T cells and IL13 (4MS)-EphA2 scFv-TanCAR Jurkat T cells were co-cultured with U87 cells at an E:T ratio of 1×10^5:1×10^5, and after 24 hours, luciferase activity was measured using a luciferase reporter assay kit. **(B)** NT Jurkat T cells, IL13 (4MS) Jurkat CAR T cells, EphA2 scFv Jurkat CAR T cells, and IL13 (4MS)-EphA2 scFv-TanCAR Jurkat T cells were co-cultured with U87 cells at an E:T ratio of 1×10^5:1×10^5, and after 24 hours, luciferase activity was measured using a luciferase reporter assay kit. **(C)** NT Jurkat T cells, IL13 (4MS) Jurkat CAR T cells, EphA2 scFv Jurkat CAR T cells, and IL13 (4MS)-EphA2 scFv-TanCAR Jurkat T cells were co-cultured with EphA2-engineered K562 target cells, IL13Rα2-engineered K562 target cells, or EphA2-IL13Rα2-engineered K562 target cells individually at an E:T ratio of 1×10^5:1×10^5, and after 24 hours, luciferase activity was measured using a luciferase reporter assay kit. Statistically significant differences are indicated: *P<0.05; **P<0.01; *** P<0.001. TanCAR stands for IL13 (4MS)-EphA2 scFv-TanCAR.

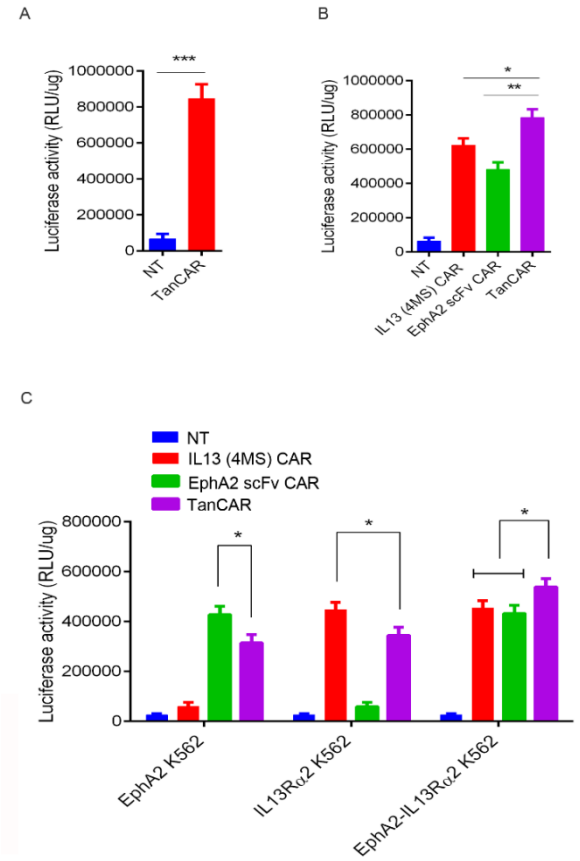

Figure S5. Functional characterization of the novel bispecific IL13 (4MS)-EphA2 scFv-TanCAR in Jurkat T cells. (A) NT Jurkat T cells and IL13 (4MS)-EphA2 scFv-TanCAR Jurkat T cells were co-cultured with U87 cells at an E:T ratio of 1×10^5:1×10^5, and after 24 hours, luciferase activity was measured using a luciferase reporter assay kit. **(B)** NT Jurkat T cells, IL13 (4MS) Jurkat CAR T cells, EphA2 scFv Jurkat CAR T cells, and IL13 (4MS)-EphA2 scFv-TanCAR Jurkat T cells were co-cultured with U87 cells at an E:T ratio of 1×10^5:1×10^5, and after 24 hours, luciferase activity was measured using a luciferase reporter assay kit. **(C)** NT Jurkat T cells, IL13 (4MS) Jurkat CAR T cells, EphA2 scFv Jurkat CAR T cells, and IL13 (4MS)-EphA2 scFv-TanCAR Jurkat T cells were co-cultured with EphA2-engineered K562 target cells, IL13Rα2-engineered K562 target cells, or EphA2-IL13Rα2-engineered K562 target cells individually at an E:T ratio of 1×10^5:1×10^5, and after 24 hours, luciferase activity was measured using a luciferase reporter assay kit. Statistically significant differences are indicated: *P<0.05; **P<0.01; *** P<0.001. TanCAR stands for IL13 (4MS)-EphA2 scFv-TanCAR.

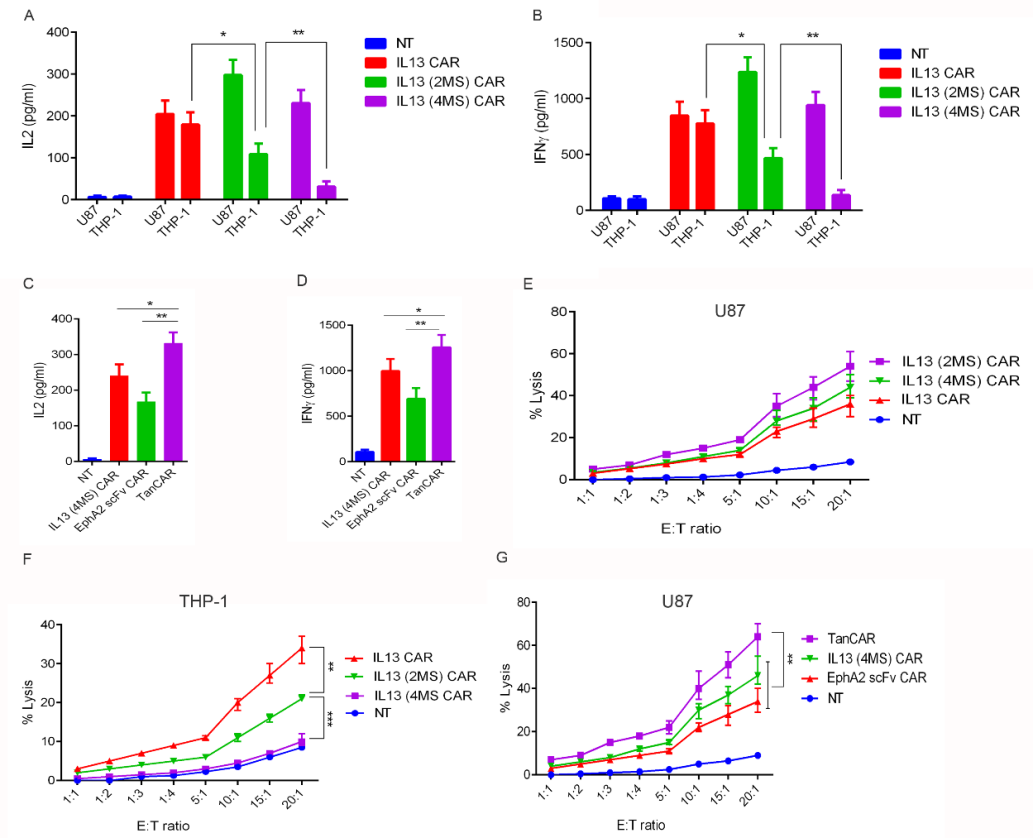

Figure S6. Functional characterization of the novel bispecific IL13 (4MS)-EphA2 scFv-TanCAR in human primary CD8+ T cells. (A) The detection of IL2 levels in the supernatants from different groups of CD8+ CAR-T cells co-cultured with U87 cells or THP-1 cells. **(B)** The detection of IFNγ levels in the supernatants from different groups of CD8+ CAR-T cells co-cultured with U87 cells or THP-1 cells. **(C)** The detection of IL2 levels in the supernatants from different groups of CD8+ CAR-T cells co-cultured with U87 cells. **(D)** The detection of IFNγ levels in the supernatants from different groups of CD8+ CAR-T cells co-cultured with U87 cells. **(E)** The measurement of the cytotoxic activity of different groups of CD8+ CAR-T cells for U87 cells by LDH level assay. **(F)** The measurement of the cytotoxic activity of different groups of CD8+ CAR-T cells for THP-1 cells by LDH level assay **(G)** The measurement of the cytotoxic activity of different groups of CD8+ CAR-T cells for U87 cells by LDH level assay. Statistically significant differences are indicated: *P<0.05; **P<0.01. TanCAR stands for IL13 (4MS)-EphA2 scFv-TanCAR.

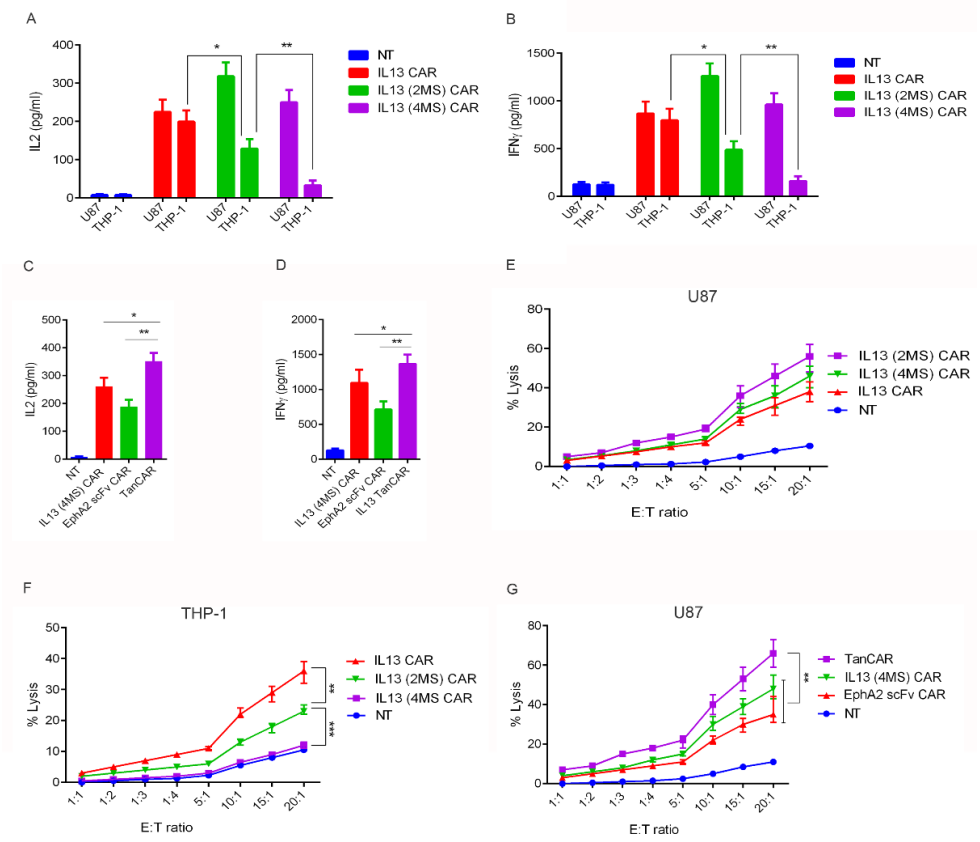

Figure S7. Functional characterization of the novel bispecific IL13 (4MS)-EphA2 scFv-TanCAR in human primary CD8+ T cells. (A) The detection of IL2 levels in the supernatants from different groups of CD8+ CAR-T cells co-cultured with U87 cells or THP-1 cells. **(B)** The detection of IFNγ levels in the supernatants from different groups of CD8+ CAR-T cells co-cultured with U87 cells or THP-1 cells. **(C)** The detection of IL2 levels in the supernatants from different groups of CD8+ CAR-T cells co-cultured with U87 cells. **(D)** The detection of IFNγ levels in the supernatants from different groups of CD8+ CAR-T cells co-cultured with U87 cells. **(E)** The measurement of the cytotoxic activity of different groups of CD8+ CAR-T cells for U87 cells by LDH level assay. **(F)** The measurement of the cytotoxic activity of different groups of CD8+ CAR-T cells for THP-1 cells by LDH level assay **(G)** The measurement of the cytotoxic activity of different groups of CD8+ CAR-T cells for U87 cells by LDH level assay. Statistically significant differences are indicated: *P<0.05; **P<0.01. TanCAR stands for IL13 (4MS)-EphA2 scFv-TanCAR.

Table S1. The primers used for generating IL13 mutants

IL13 SfuI Forward	ATTCGAATCCCCAGGCCCTGTGCCTCC
IL13 NheI reverse	AGCTAGCGTTGAACTGTCCCTCGCGAA
IL13 E13K Forward	ccctctacagccctcaggAgactcattgaggagctggtc
IL13 E13K reverse	gaccagctcctcaatgagtcTcctgagggctgtagaggg
IL13 R109K forward	catttaaagaaacttttttAAAgagggacagttcaactga
IL13 R109K reverse	tcagttgaactgtccctcTTTaaaaagtttctttaaatg
IL13 R66D.S69D Forward	gccatcgagaagacccagGACatgctgGACggattctgcccgcacaag
IL13 R66D.S69DReverse	cttgtgcgggcagaatccGTCcagcatGTCctgggtcttctcgatggc

Table S2. The primers used for RT-qPCR analysis

Primers	**Primer Sequence (5'-3')**
IL13Rα1 Forward	TGTGCAATGGGAGAATCCACA
IL13Rα1 Reverse	TGCGACGATGACTGGAACAA
IL13Rα2 Forward	ACCTTTGCCGCCAGTCTATC
IL13Rα2 Reverse	GGTCTTCACCTTCCCAGCAT
EphA2 Forward	TGGCTCACACACCCGTATG
EphA2 Reverse	GTCGCCAGACATCACGTTG
GAPDH Forward	TGGTGAAGACGCCAGTGGA
GAPDH Reverse	GCACCGTCAAGGCTGAGAAC